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A Bt TRANSGENE REDUCES HERBIVORY AND ENHANCES FECUNDITY IN WILD SUNFLOWERS

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Abstract. Gene flow from transgenic crops can introduce novel traits into related species, but the ecological importance of this process is unknown. Here, we report the first empirical evidence that wild plants can benefit from a bacterial transgene under uncaged, natural conditions. Cultivated sunflower (*Helianthus annuus*) is known to hybridize frequently with wild sunflower (*H. annuus*) in the western and midwestern United States. We studied a crop-developed *Bacillus thuringiensis* (Bt) transgene, *cry1Ac*, in backcrossed wild sunflower populations. Lepidopteran damage on transgenic plants was strongly reduced relative to control plants at our two study sites, while damage by several weevil and fly species was unaffected. Our results suggest that reduced herbivory caused transgenic plants to produce an average of 55% more seeds per plant relative to nontransgenic controls at the field site in Nebraska. A similar but nonsignificant trend was seen at the site in Colorado (14% more seeds per plant). In a greenhouse experiment the transgene had no effect on fecundity, suggesting that it was not associated with a fitness cost. If Bt sunflowers are released commercially, we expect that Bt genes will spread to wild and weedy populations, limit damage from susceptible herbivores on these plants, and increase seed production when these herbivores are common.

Key words: Bt transgene; ecological effects; fecundity; fitness benefits and fitness costs; gene flow, ecological and evolutionary effects; Helianthus annuus; *herbivore damage; hybridization; risk assessment; sunflower, wild; transgenic crops.*

INTRODUCTION

Worldwide, many cultivated plants hybridize spontaneously with wild or weedy relatives (Small 1984, Ellstrand et al. 1999). In the United States, for example, this occurs in more than twenty species, including sunflower, sorghum, squash, canola, rice, sugar beet, poplar, turf grasses, and forage grasses (NRC 2000). In addition, many crops can become naturalized and persist as feral weed populations (Ellstrand et al. 1999). Thus, transgenes conferring novel traits that enhance survival and reproduction may inadvertently disperse from cultivated plants to wild or weedy populations that lack these traits. In the short term the spread of transgenic herbicide resistance is likely to pose challenges for controlling weeds and unwanted ''volunteer'' crop plants (Snow et al. 1999, Hall et al. 2000). Over the longer term we need to know whether the spread of transgenes coding for other fitness-related traits could exacerbate weed problems in agricultural settings and affect the population dynamics of wild relatives in unmanaged areas (Snow and Morán Palma 1997, NRC 2000). This raises fundamental questions about the extent to which herbivores, diseases, and stressful abiotic conditions regulate populations of wild and weedy plants. With regard to herbivores, studies of both native and exotic species suggest that herbivores can have a dramatic impact on plant population dynamics (e.g., Crawley 1997, Rees and Paynter 1997). However, little is known about how herbivores affect the population dynamics of wild or weedy relatives of cultivated plants. Without findings from carefully designed field experiments, it is difficult to estimate the net impact that herbivores have on plant survival, growth rates, and seed production.

Sunflower (*Helianthus annuus*) is an attractive study system because the self-incompatible wild sunflower (also *H. annuus*) is an agricultural weed that hybridizes easily with the crop (Arias and Rieseberg 1994, Snow et al. 1998). Wild sunflower is common along field and

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road margins near domesticated plants, as well as in other agricultural and natural areas in the western and midwestern United States (Heiser 1954). Previous studies have demonstrated crop–wild hybridization rates as high as 40% on wild plants that occur adjacent to the crop, with small but detectable levels of hybridization as far as 1000 m away (Arias and Rieseberg 1994). Crop genes can easily backcross into wild sunflower populations (Whitton et al. 1997). In North Dakota (USA) and Canada, for example, Linder et al. (1998) reported that every wild plant sampled from areas where sunflower was cultivated had one or more crop-specific DNA markers.

Wild sunflower populations host many of the same herbivores and diseases as cultivated sunflower (Seiler 1992), so transgenes coding for novel traits such as insect resistance have the potential to benefit wild genotypes. Seed companies have field tested sunflower varieties with transgenic resistance to moth and weevil larvae, which are economically important crop pests, although none have been commercialized to date. Our study involved the Bt protein Cry1Ac, which is toxic to lepidopteran species but is not expected to affect other insect taxa (Estruch et al. 1997). Ingesting a very small amount of Bt toxin (parts per 10^9) typically causes susceptible insects to either stop feeding and die within a few days (Estruch et al. 1997) or move to a nontoxic host plant (Davis and Onstad 2000).

The goals of this study were to determine (1) whether a Bt transgene was effective at reducing herbivory in backcrossed wild sunflowers under uncaged field conditions, (2) whether the transgene was associated with increased fecundity in wild sunflowers in the field, and (3) whether the transgene was associated with any inherent fitness cost or benefit when plants were grown in a greenhouse, without being exposed to herbivores.

Materials and methods

Experimental approach

Determining the ecological effects of pre-commercial transgenes is inherently difficult due to biosafety and regulatory concerns. Uncaged plants must be exposed to natural levels of insect damage and cross-pollination, yet dispersal and persistence of the transgene(s) must be prevented. To simulate the effects of introgression of a Bt transgene from the crop and avoid the problem of pollen dispersal, we used specific genotypes of wild sunflower to create BC_1 progeny that were male sterile and segregated for the presence or absence of the Bt transgene. Among BC_1 progeny without the transgene, we also compared plants with and without pollen to determine the effects of male sterility on herbivory and seed production (some seed predators feed on pollen [Delisle et al. 1989, Korman and Oseto 1989] and might avoid male-sterile plants). Field experiments were carried out in Nebraska and Colorado, USA, to test for fitness effects of the transgene under natural conditions. A greenhouse experiment was used to determine whether the transgene or male sterility affected plant fecundity in the absence of herbivores.

Crosses for BC_1 progeny

We used a transgenic cultivated line (CMS-PET1) with cytoplasmic male sterility as the original female parent. Nearly all cultivated sunflower in the United States is sold as hybrid seed produced by inbred plants with this type of male sterility. The transgenic line was developed for potential commercialization and had a single copy of a plant-optimized *cry1Ac* gene from *Bacillus thuringiensis* (''Bt'') fused to a constitutive promoter (modified CaMV 35S), and lacked a selectable marker. Cytoplasmic male sterility is inherited maternally, so progeny produced from this transgenic parent were male sterile unless sired by wild plants with nuclear alleles for fertility restoration. Most wild plants have one or more alleles that restore male fertility, but we were able to select wild pollen donors for the F_1 and BC_1 generations that were heterozygous for restorer alleles or lacked them entirely. Heterozygous pollen donors produced both male-sterile and male-fertile BC_1 offspring, but, to avoid the spread of this experimental transgene via pollen, male-fertile plants that were Bt positive were not used in the field experiment. We used a total of ~15 wild genotypes from Ogallala, Nebraska, as pollen donors. This relatively large number of sires minimizes the probability that our results were influenced by particular wild genotypes. BC_1 seeds from all crosses were pooled into a single group of progeny for the field and greenhouse experiments. Within this group of progeny, 44% were male sterile and 49.3% were Bt positive (N = 740 plants). The 1:1 segregation of Bt-positive and Bt-negative genotypes is consistent with the expectation that transgenes are inherited in the same manner as naturally occurring genes (e.g., Snow et al. 1999, Halfhill et al. 2002).

Field Experiments

Experimental protocol.—BC_1 seeds were germinated in petri dishes, sown in flats, and seedlings were transplanted to two field sites ~245 km apart in May 1999. One plot was a grassy area at Cedar Point Biological Station, Ogallala, Nebraska, and the other was an agricultural field in Burlington, Colorado. Both sites were tilled prior to planting. The Colorado plot received herbicide and nutrient applications typical of cultivated sunflower fields. The Nebraska plot was similar to a natural disturbance in grassland and was not weeded. To account for possible microsite differences, we subdivided the plots to include six blocks in Nebraska and four blocks in Colorado. BC_1 and wild seedlings were planted alternately, with either 25 cm (Nebraska) or 30 cm (Colorado) between plants in the block. Wild plants were included to standardize competitive effects and provide pollen for cross-fertilization. The treatment

types of the BC_1 plants (i.e., with or without Bt, with or without pollen) were not known at the time of planting, so we assumed that the locations of plants within each treatment group were random.

Few plants died, and the Colorado plants grew to be about twice as large as those in Nebraska. These experiments were carried out under USDA-APHIS (Animal and Plant Health Inspection Service) Permits 99-096-01N and 99-095-07N. All wild and BC_1 seed heads were collected, and any sunflower seedlings that appeared at the sites after tilling in 2000 and 2001 were destroyed.

Identification of transgenic plants.—Prior to flowering, we collected two 7-mm-diameter leaf disks from each BC_1 plant to determine which plants were transgenic. Plants that lacked a PCR-based marker for the transgene construct and did not produce Bt toxin (determined by enzyme-linked immunosorbent assays [ELISAs]) were designated as ''Bt negative'' (Bt−), while those that had the marker and produced the toxin were designated ''Bt positive'' (Bt+). We bagged the first flower head of each plant before it opened to check for male sterility. Bt-positive plants that produced pollen were destroyed, and bags on other plants were removed to allow pollination. Treatment groups were designated Bt positive and male sterile (Bt+/male sterile), Bt−/male sterile, and Bt−/male fertile ($N = 60$ plants per treatment in Nebraska and 50 per treatment in Colorado).

Herbivore damage and plant fecundity.—Herbivores of wild sunflower produce several characteristic types of damage (Cummings et al. 1999, Pilson 2000). In Nebraska only we censused stem tunnel holes produced by first-generation *Suleima helianthana* (on 1 July, prior to flowering) and numbers of flower heads clipped by the weevil *Haplorhynchites aeneus*. We did not quantify leaf damage, which was negligible at both sites. Seed heads (i.e., inflorescences with mature seeds) were collected prior to seed dispersal and stored for later analyses. In Colorado, ~4% of the seeds were lost prior to collection but the percentage lost did not differ among experimental groups. For each plant, we recorded the proportion of heads with damage by *Plagiomimicus spumosum*, which leaves a characteristic ''bald spot'' on the seed head. Each head was then dissected and we recorded the number of good seeds, as well as categorical estimates of seeds damaged by *Isophrictis similiella*, *Smicronyx fulvus*, *S. sordidus* (Nebraska only), *Cochylis hospes*, and *Neolasioptera helianthi* (Nebraska only). Total plant fecundity was calculated by adding the number of good seeds per head for all heads on a plant. We also report the total number of inflorescences per plant, which can influence the plant's male reproductive success. Male reproductive success can also be affected by other characteristics, however, such as pollen production, pollen viability, pollinator preferences, and flowering times. To characterize flowering times, we recorded the first day of anthesis for each BC_1 plant.

Statistical analyses.—Data on stem, head, or seed damage, number of heads per plant, number of seeds per plant, and days to flowering were analyzed using the GLM or CATMOD procedures of SAS (SAS 1990), for continuous and categorical data, respectively. Analyses of each site separately included block and treatment (treatments were Bt+/male sterile, Bt−/male sterile, or Bt−/male fertile). The block × treatment interaction was never significant ($P > 0.50$ in all cases), and was dropped from the analyses reported here. The analyses of both sites together included site (Nebraska or Colorado), block(site), treatment, and the site × treatment interaction. Block and site, and interactions involving these effects, were considered random effects, and significance tests were constructed accordingly. Although the site effects in the combined analyses were generally significant, in none of the analyses was the interaction between study site and treatment statistically significant ($P > 0.70$ in all cases), indicating that the transgene had similar effects on insect damage and plant fecundity at the two sites. To test for the effect of the transgene on damage and fecundity, in each of these analyses we examined planned contrasts between BC_1 plants that were Bt+/male sterile and Bt−/male sterile. Similarly, to test for the effect of male fertility we examined planned contrasts between BC_1 plants that were Bt−/male sterile and Bt−/male fertile. When necessary the response variables were transformed (square or cube root, or log, depending on the variable) to meet assumptions of the analyses.

Greenhouse experiment

The purpose of this experiment was to test for any inherent differences in the fecundity of transgenic versus nontransgenic plants when grown in the absence of herbivores. This experiment was not carried out in the field because of the difficulties of excluding insect herbivores and carrying out hand pollinations on every flower head over a period of 1–2 months. Although the greenhouse environment is very different from field conditions, we expected major genetic differences among treatments would be detectable. In addition, we subjected the plants to nutrient and drought stress to test for genotype-by-environment interactions.

We used a three-way factorial design to examine effects of the transgene, male sterility, growing conditions, and interactions among these factors on lifetime seed production. Because this experiment was done in the greenhouse, the Bt+/male fertile plants could be retained. In November 1999, we grew ~80 BC_1 plants in each of three growing conditions in a greenhouse with high-intensity halogen lights in Columbus, Ohio. Each plant was grown in a 30-cm-diameter pot filled with standard potting soil. Control plants were watered

TABLE 1. Effects of the Bt transgene on the amount of insect damage and lifetime fecundity of BC_1 sunflowers (*Helianthus annuus*) at the study sites in Nebraska and Colorado, USA, analyzed separately and together.

	Nebraska			Colorado			Both sites combined
Herbivore species or fitness trait	Bt+/male sterile	Bt−/male sterile	*P*	Bt+/male sterile	Bt−/male sterile	*P*	*P*
Lepidopteran damage†							
Isophrictis similiella (Gelechiidae)‡	0.037	0.465	0.0001	0.003	0.189	0.0001§	0.0001
Cochylis ssp. (Tortricidae; banded sunflower moth)‡	0.007	0.074	0.006	0.007	0.056	0.04§	0.001§
Suleima helianthana (Tortricidae; sunflower bud moth) (1st generation: proportion stems with damage; categorical data)	0.100	0.550	0.001	no data	no data	⋯	⋯
Plagiomimicus spumosum (Noctuidae) (proportion heads with damage)	0.008	0.110	0.011§	0.082	0.113	NS§	0.024§
Damage by other insects							
Haplorhynchites aeneus (Attelabidae; sunflower head-clipping weevil) (proportion heads clipped)	0.072	0.077	NS	no data	no data	⋯	⋯
Smicronyx fulvus (Curculionidae; red sunflower seed weevil)‡	0.249	0.179	NS	0.150	0.204	NS	NS
Smicronyx sordidus (Curculionidae; gray sunflower seed weevil)‡	0.040	0.034	NS	no data	no data	⋯	⋯
Neolasioptera helianthi (Cecidomyiidae; sunflower seed midge)‡	0.387	0.357	NS	no data	no data	⋯	⋯
Plant fecundity							
Date of anthesis of first inflorescence (no. days after the first BC_1 plant flowered at each site)	21.7	20.5	NS	16.0	17.8	NS	NS
Inflorescences per plant	6.1	5.0	NS	15.0	12.4	NS	0.051
Inflorescences with mature seeds per plant	5.2	4.1	NS	10.5	8.7	0.045	0.036
Seeds/mature inflorescence	176	152	NS	212	218	NS	NS
Seeds per plant	909	588	0.054	2077	1814	NS	0.037‖

Notes: For Nebraska there were 58–60 plants/treatment and for Colorado, 47–50 plants per treatment. Data shown are untransformed means. *P* values reported here are for planned contrasts between the Bt+/male sterile and Bt−/male sterile treatments following ANOVA; NS indicates $P > 0.10$.

† These lepidopteran herbivores specialize on some species within the genus *Helianthus*, and are most common on *H. annuus; Cochylis hospes* and *Suleima helianthana* can be economically important crop pests. We were unable to census damage by the sunflower moth, *Homoeosoma electellum* (Pyralidae), another seed-feeding crop pest that was common at our study sites.

‡ Damage on each inflorescence was quantified as follows: 0 = 0 seeds damaged; 1 = 1–30 seeds damaged; 2 = >30 seeds damaged. For each plant the mean value over all that plant's inflorescences was used in ANOVAs using GLM procedure of SAS (SAS Institute 1990).

§ Male-fertile plants had significantly more damage than male-steriles ($P < 0.05$). For *P. spumosum* in Colorado this effect was seen for the mean amount of damage/plant, but not for the frequency of heads with damage.

‖ In the combined data set, when the number of inflorescences was included as a covariate in an ANCOVA of seeds per plant, the *P* value for the planned contrast testing the Bt effect was 0.4350. When the number of seeds per mature inflorescence was included as a covariate, the *P* value for the planned contrast testing the Bt effect was 0.0106.

daily as needed and fertilized with a standard amount of slow-release fertilizer. Water-stressed plants were fertilized in the same way, but were subjected to repeated wilting (once or twice per week for 12 weeks). Nutrient-stressed plants were watered daily as needed but did not receive any soil nutrients to supplement those in the potting soil. As in the field experiments, we used PCR and ELISA methods on leaf disk samples to determine which plants were Bt positive, and we observed anthers to determine which plants were male sterile. Sample sizes are lower for stressed plants (Fig. 2) because PCR and ELISA tests were sometimes inconclusive. To facilitate cross-pollination, captive bumblebees were used to supplement hand pollinations. Unpollinated florets were easily identified by their exserted stigmas and were pollinated by hand on a daily basis to ensure maximum seed set. Individual stigmas are receptive for several days, and the daily checks for unpollinated stigmas made it unlikely that any florets were overlooked. When seeds were mature, we counted numbers of inflorescences and seeds per plant. The plants in this experiment grew vigorously and reached sizes that were similar to our field-grown BC_1 plants in Nebraska.

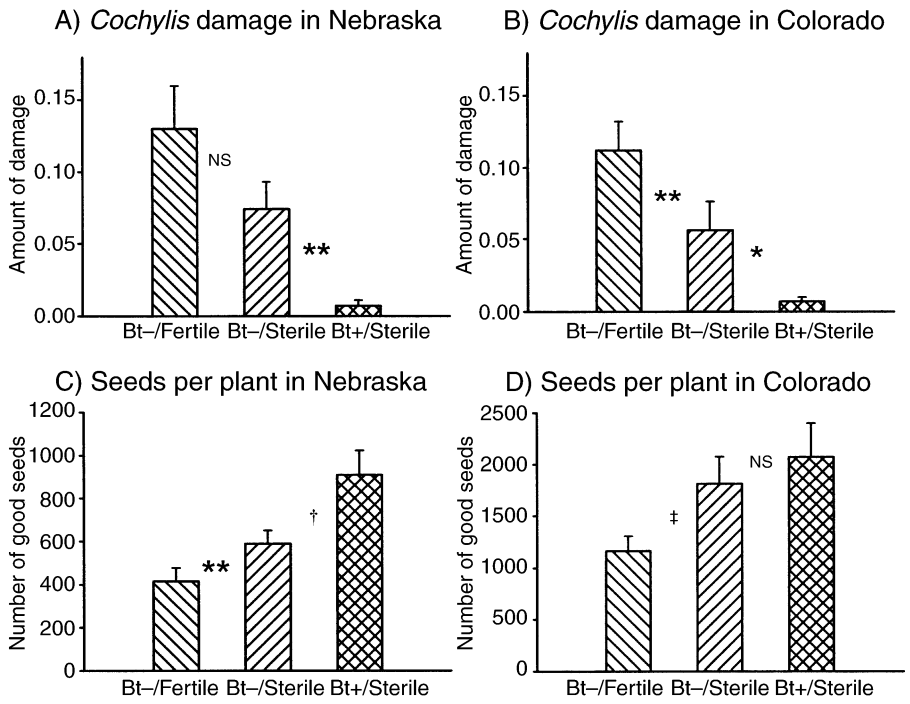

FIG. 1. Effects of the Bt transgene and male sterility on relative amounts of sunflower seed damage by *Cochylis* moth species and the number of good seeds (undamaged) per plant in Nebraska and Colorado (USA). Untransformed means and 1 SE are shown; $N = 58$–60 plants in Nebraska, $N = 47$–49 plants in Colorado. Levels of statistical significance are based on planned contrasts between adjacent treatment means (see Table 1 for details, including methods for reporting damage levels).

*$P < 0.05$; **$P < 0.01$; †$P = 0.054$; ‡ $P = 0.077$; NS, $P > 0.10$.

RESULTS AND DISCUSSION

Field experiments

Transgenic resistance to lepidopterans appears to be a dominant trait because BC_1 plants that were hemizygous for the *cry1Ac* gene had very low levels of lepidopteran damage at both field sites (Table 1, Fig. 1). Halfhill et al. (2002) also reported strong suppression of target herbivores in hemizygous BC_1 progeny from wild *Brassica rapa* and cultivated *B. napus* with a *cry1Ac* transgene. Further experiments would be useful to determine whether plants that are homozygous for the transgene produce greater concentrations of Bt toxin and experience less herbivory than hemizygous plants.

Flowering times did not differ between transgenic plants and nontransgenic controls at either site (Table 1), so temporal differences between treatments were not likely to affect exposure to herbivores. In Nebraska, stem damage by first-generation sunflower bud moth larvae (*Suleima helianthana*) was about five times more common in nontransgenic controls as compared to transgenic plants (Table 1; this type of damage was not examined in Colorado). Likewise, larvae of *Isophrictis similiella* and *Cochylis* spp. (*C. hospes* and *C. arthuri*) destroyed more seeds on control plants than on transgenic plants at both sites (Table 1, Fig. 1). Flower head damage by the moth *Plagiomimicus spumosum* was more common in nontransgenic plants at both sites, but this effect was only marginally significant in Colorado ($P < 0.10$, Table 1). These three lepidopteran seed predators generally caused more damage on male-fertile than on male-sterile plants (Table 1, Fig. 1), indicating that the benefits of Bt may be underestimated in male-sterile plants. The Bt toxin had no effect on amounts of damage caused by four non-lepidopteran species (head-clipping weevils, red and gray seed weevils, and the sunflower seed midge; Table 1), as expected. Although some of these species are negatively affected by competition with lepidopterans (M. J. Paulsen, and D. Pilson, *unpublished data*), they did not cause more damage on Bt plants than on controls.

Transgenic plants produced an average of 55% more seeds per plant than nontransgenic controls in Nebraska and 14% more seeds per plant in Colorado (Table 1, Fig. 1). In separate ANOVAs for each site, this effect was significant at $P < 0.054$ for Nebraska and was not significant for Colorado ($P = 0.262$). This difference in significance could be due to lower statistical power for the Colorado data (which had smaller sample sizes), differences in herbivore pressure at the two sites, and/or the fact that nonlocal Nebraska genotypes were grown in Colorado, whereas local genotypes were grown in Nebraska. In any event, the same trend was

seen at both sites (Fig. 1). An ANOVA that included both sites showed that the interaction between site and the Bt transgene was not statistically significant, so it is appropriate to examine the main effects of the transgene (Table 1). In this ANOVA, we found that transgenic plants produced significantly more inflorescences ($P < 0.051$), more inflorescences that produced mature seeds ($P < 0.036$), and more viable seeds per plant ($P < 0.037$) than nontransgenic controls (Table 1). Among the nontransgenic plants, seed production was significantly lower in male-fertile plants as compared to male-sterile plants (Fig. 1, Table 1), presumably because they experienced greater lepidopteran damage than male-sterile plants.

To explore the underlying causes of the fecundity advantage of transgenic plants, we used the number of inflorescences per plant as a covariate in an ANCOVA (Table 1: 11 footnote). This caused the significant Bt effect to disappear, suggesting that differences in the number of seeds per plant were largely due to differences in the numbers of inflorescences. This may be due to greater root and stem damage in nontransgenic plants, resulting in smaller plants with fewer inflorescences. Although we did not attempt to comprehensively sample internal damage, *I. similiella* is known to feed within the stem (Charlet 1983), *Eucosma womonana* feeds on the roots (Rogers 1985), and both can be common at the Nebraska study site (D. Pilson and M. Paulsen, personal observation). Furthermore, second-generation *S. helianthana* attacks flower buds, causing them to abort before opening, and this may result in plants with fewer inflorescences.

It is possible that reduced herbivore feeding on transgenic plants was associated with lower levels of induced defense, which might afford a fitness benefit. For example, a study of wild radish showed that the induced production of anti-herbivore compounds caused plants to produce fewer seeds (Agrawal 1998). Lower herbivore damage in Bt plants might also be associated with reduced disease transmission. However, disease symptoms were mild in our study and were not affected by the transgene (D. Pilson and M. J. Paulsen, *unpublished data*). Another possibility is that the fecundity advantage we observed was due to other unanticipated mechanisms, as discussed below.

In any study of a single transformation event, it is not clear whether phenotypic effects (e.g., greater fecundity) are caused by the transgenic construct or by other mechanisms, such as position effects (e.g., linkage to other crop genes) or pleiotropy. Thus, it is useful to determine whether effects associated with the Bt transgene can occur in the absence of lepidopteran herbivores. We performed a greenhouse experiment to examine this possibility, while recognizing there are many biotic and abiotic differences between field and greenhouse conditions.

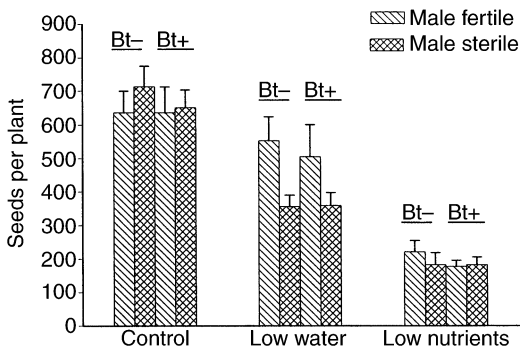

FIG. 2. Effects of the Bt transgene and male sterility on the seed production of BC_1 sunflower plants grown under three conditions (Control, Low water, Low nutrients) in a greenhouse. Data are means and 1 SE. The only significant effects in a three-way ANOVA were growing condition ($P < 0.0001$) and the interaction between growing condition and male fertility ($P < 0.0042$; not significant in a similar analysis of the number of flower heads per plant). Sample sizes (from left to right) were: Control—19, 24, 16, and 27 plants per treatment; Low water—17, 27, 12, and 24 plants; and Low nutrients—14, 11, 14, and 12 plants.

Greenhouse experiment

The Bt transgene had no effect on the number of inflorescences or seeds per plant in the greenhouse, regardless of whether the plants were grown under water-stressed, nutrient-stressed, or non-stressed conditions, and regardless of whether they were male fertile or male sterile (Fig. 2). This indicates that the transgene was not associated with an inherent fitness cost or benefit. It would be preferable to employ a wider range of growing conditions and several transgenic events in this type of study, but our results suggest that the fecundity advantage of transgenic plants in the field was due to protection from lepidopteran herbivores. The greenhouse experiment also showed that the fecundity of male-fertile plants was similar to that of male-sterile plants, except for plants in the water-stressed treatment. In that treatment, male-sterile plants produced fewer seeds than male-fertile plants, perhaps due to decreased ovule fertilization or increased embryo abortion.

Conclusions

This study shows that a single Bt transgene can have dramatic effects on herbivory and fecundity in BC_1 wild sunflowers, which is consistent with studies of Bt crop plants (e.g., Peferoen 1997, Barry et al. 2000). The magnitude of the fecundity benefit is likely to vary among sites and seasons, depending on the prevalence of susceptible herbivores. We found that this benefit can be as high as a 55% increase in seed production, and possibly as high as a *doubling* of seed production if nontransgenic male-fertile plants are compared to the transgenic male-sterile plants. The higher estimate is based on the finding that male-fertile plants had more lepidopteran damage and fewer seeds per plant than male-sterile plants in the field. A likely reason for this

discrepancy is that pollen-producing plants were more attractive to lepidopteran herbivores (Delisle et al. 1989, Korman and Oseto 1989).

The fecundity benefit we observed suggests that selection favoring an increase in the frequency of a Bt transgene has the potential to be quite strong. A more complete measure of plant fitness would include estimates of male reproductive success, which was not addressed in this study. It is possible that transgenic plants produced more pollen per anther or were more attractive to pollinators relative to control plants, or that they were *less* successful for unknown reasons, but we have no reason to expect the latter. If herbivores cause less damage to wild genotypes than to BC_1 sunflowers, it is possible that the fecundity advantage associated with Bt would diminish with subsequent generations of backcrossing (Cummings et al. 1999). Even if this occurs, we may have underestimated the fecundity advantage of Bt in this study, and we have observed higher levels of lepidopteran damage on wild plants in other years (Pilson 2000; D. Pilson and M. J. Paulsen, *unpublished data*). Therefore, we expect that subsequent generations of Bt wild plants would produce more seeds per plant than nontransgenic individuals in many locations and growing seasons. Because the rate of spread of an allele is controlled largely by its selective advantage, a Bt transgene could spread quickly across wild sunflower populations. In addition, wild populations that occur near cultivated Bt sunflower would be exposed to repeated episodes of gene flow from the crop. The rapid spread of Bt transgenes could potentially lead to a reduction in population size of susceptible, native lepidopterans that feed on wild sunflower. It is possible that specialist herbivores would eventually evolve resistance to transgenic Bt toxins, but this has not been reported yet in target pests of transgenic Bt cotton or corn (e.g., Carriere et al. 2001).

In summary, our study is the first to demonstrate that a transgene derived from a crop has the potential to increase the fitness of wild plants, and thus increase in frequency in wild populations. The large effect of a constitutively produced Bt toxin on lepidopteran damage, and consequently also on fecundity, is noteworthy and suggests that lepidopteran herbivory within stems, roots, and flower heads can be extensive. We are not aware of other cases in which a gene derived from a crop, transgenic or otherwise, has been clearly shown to increase the fitness of wild plants under realistic field conditions. For transgenes to have important ecological effects in wild populations, they must also alter interactions between the wild plant and its biotic and abiotic environment. A *cry1Ac* gene that becomes common in wild sunflower populations will clearly have negative effects on the suite of native lepidopteran herbivores that use wild sunflowers as their primary host plant. Moreover, other types of Bt genes could potentially reduce populations of coleopteran and dipteran herbivores.

Next, we plan to determine whether transgenic wild sunflowers that produce more seeds are likely to create larger populations, more populations, and/or more extensive seed banks. Experimental manipulations of local population dynamics and models of metapopulation dynamics are needed to understand these processes. In addition, to gain a better understanding of how lepidopteran herbivores affect wild sunflower populations, experiments similar to those we have reported here should be repeated over several study sites and seasons using advanced backcrossed generations. Fitness studies are an essential first step in understanding the ecological and evolutionary effects of gene flow because it is important to know the magnitude of presumed fecundity effects of a given transgene. This knowledge, together with an evaluation of the ecological effects of transgenes, is critical for biosafety risk assessments.

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